Application of the FAIMS Pro Duo Interface for Selective Detection of Lower Abundance Lipid Classes at Analytical Flow Rates

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ABSTRACT

Purpose: To demonstrate the application of field-asymmetric ion mobility spectrometry at analytical flow rates for selective analysis of different lower-abundant lipid classes from untargeted lipidomics experiments.

Methods: Several commercially available lipid extracts were analyzed at 260 µL/min using a Thermo Scientific™ Vanquish™ UHPLC system coupled with a Thermo Scientific™ FAIMS Pro Duo interface on the front-end of a Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer. The data were processed using Thermo Scientific™ Freestyle™ 1.8 SP2 and LipidSearch™ 4.2 software.

Results: Using parameters obtained from initial method optimization on lipid class standards, the LC-MS analysis of multiple lipid extracts was carried out at different CV settings, leading to the selective detection of lipid classes. Examples of complete resolution of isobaric lipid species using the FAIMS Pro Duo interface are shown here. Use of the FAIMS Pro Duo interface enabled increased lipid class annotation at their respective optimal CV values in several cases, when compared to LC-MS analysis without FAIMS.

INTRODUCTION

The FAIMS Pro Duo interface operates as a tunable ion filter providing an additional separation dimension between LC separation and MS measurement. It reduces chemical noise and matrix interferences, resulting in improved robustness, simplification of the sample. In many assays, better limits of detection/quantification can be observed as a result. The field-asymmetric ion mobility spectrometry (FAIMS) separation is based on a combination of charge state, shape and size of gas phase ions. Here, we present the use of FAIMS technology for selective gas-phase enrichment of specific lipid classes at analytical flow rates from untargeted lipidomics experiments, with MS data acquired on a Thermo Scientific™ Orbitrap ID-X™ Tribrid mass spectrometer.

MATERIALS AND METHODS

Sample Preparation

Initial optimization of FAIMS Compensation Voltage (CV) values were performed with representative lipid class standards contained in the SPLASH™ LipidoMix™ (Avanti Polar Lipids, Alabaster, AL, USA). For LC-MS experiments, bovine liver, bovine heart total lipid extracts, soy polar lipid extract and Porcine brain total lipid extract (Avanti Polar Lipids, Alabaster, AL, USA) were used as representative extract samples of different origin and relative lipid contents. The extracts (25 mg/mL in chloroform) were diluted 1:100 in a mixture of 50:50 acetonitrile/isopropanol containing 1:100 diluted SPLASH™ LipidoMix™. Samples were placed in low volume glass autosampler vials and sealed using vial caps with PTFE-only septa. The vials were kept at 15 °C in the autosampler prior to and during analysis.

LC-MS Method

The LC-MS analyses were performed on a Vanquish Flex Binary UHPLC system coupled to an Orbitrap ID-X Tribrid MS. Chromatographic separation was achieved using the gradient conditions shown in Table 1. The mobile phases consisted of 60:40 acetonitrile/water for mobile phase A and 90:10 isopropanol/acetonitrile for mobile phase B, with both containing 10 mM ammonium formate and 0.1% formic acid. The separation used a Thermo Scientific™ Accucore™ C30 column (2.1x150 mm, 2.6 µm) operated at 45 °C with a flow rate of 260 µL/min. The injection volume was 2 µL.

Mass spectral data were collected with the source conditions reproduced in Table 2, both with and without the use of the FAIMS Pro Duo interface. Positive and negative ion mode data were acquired separately with data-dependent MS^n experiments to characterize eluting lipids, and in the case of FAIMS experiments, used a single CV value per analysis.

Table 1. HPLC Gradient

Time (min)	%B
0.0	30
2.0	43
2.1	55
12.0	65
18.0	85
20.0	100
25.0	100
25.1	30
31.0	30

Data Analysis

Raw data were analyzed using FreeStyle 1.8 SP2 software to analyze CV scan data and LipidSearch 4.2 software to annotate lipids from LC-MS runs.

Table 2. Source and MS method parameters used in the LC-MS analysis.

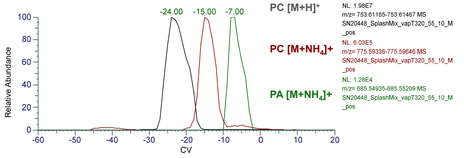
Optamax NG HESI Source		Orbitrap ID-X MS and FAIMS Pro Duo	
Spray voltage	+3.25/–3.0 kV	MS1/MS2 Resolution	120,000 / 30,000
Cap. Temp	300 °C	MS1 Mass range	200–1700 Da
Vap. Temp	320 °C	Cycle time	1.5 s
Sheath Gas	55	RF Lens	40.0
Aux Gas	10	FAIMS Resolution Setting	Normal Resolution
Sweep gas (without FAIMS)	1	Background Exclusion from Solvent blank	Top 100 ions from averaged spectrum
Sprayer position (without – with FAIMS)	1.2, M/L, center – 0.75, M, center	MS^n triggers	CID-MS2 on *m/z* 184 ion for PC; CID-MS3 on NL(FA) from TG

RESULTS

CV Value Optimization for lipid class standards

For the optimization of CV values, SPLASH LipidoMix was T-infused into the eluent post-column and data were collected from the Instrument Tune window using the 'CV Scan' feature in FullScan mode, with CV steps of 1 V from –60 to +20 V for positive ESI mode and –20 to +60 V for negative ESI mode. Three representative CV plots are depicted in Figure 1 for PC(15:0-18:1(d7)) $[M+H]^+$ and $[M+NH_4]^+$, as well PA(15:0-18:1(d7)) $[M+NH_4]^+$ in positive ESI mode, showing their differentiation.

Figure 1. CV Plots for detected ions of lipid standards PC(15:0-18:1(d7)) and PA(15:0-18:1(d7)) from ESI(+) data at 260 µL/min showing different CV optima depending on lipid class and adduct ion.



In the optimization, several parameters such as eluent flow rate, HESI probe position, probe temperature and vaporizer gas settings were varied to evaluate their respective impact on CV optima.

As seen in Figure 2 for a subset of lipids in negative ESI mode, eluent flow rate was found to have a significant effect on CV optima for the monitored lipid standards, and notably, the separation between CV optima was more distinct at 260 µL/min than at lower flow rates (1 or 100 µL/min). The impact of mobile phase composition (as a function of %B) was also investigated, as previous work has shown the addition of solvent vapors acting as gas modifiers to be an important factor at high flow rates,[1,2] however no significant shift in CV optima was observed when varying %B from 30 up to 100. This is possibly because the largest change was previously observed at lower % organic composition of the mobile phase, whereas the initial gradient conditions of 30 %B already contain 72% of organic solvents.

Figure 2. Flow rate dependence of CV optima in negative ESI mode shown for several lipid standards, with CV optima generally decreasing in voltage with increased flow rates.

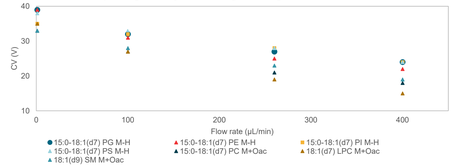


Table 3. Determined CV optima (V) for detected ions of the SPLASH LipidoMix lipid class standards at 260 µL/min under the source conditions noted in Table 2.

Lipid	RT (min)	$[M+H]^+$	$[M+NH_4]^+$	$[M+Na]^+$	$[M-H]^-$	$[M+OAc]^-$
15:0-18:1(d7) PC	9.7	-24		-15		24
18:1(d7) LPC	3.2	-25		-11		24
15:0-18:1(d7) PE	10.2	-6		-28	29	
18:1(d7) LPE	3.4	9		-4	29	
15:0-18:1(d7) PG	8.8		-20	-13	29	
15:0-18:1(d7) PI	8.5	-18	-18		29	
15:0-18:1(d7) PS	8.7	-4			33	
15:0-18:1(d7) PA	9.7		-7			
15:0-18:1(d7)-15:0 TG	20.9		-14	-15		
15:0-18:1(d7) DG	13.7		-9	-5		
18:1(d9) SM	8.8	-30			24	
18:1(d7) Chol Ester	21.9		-11			

In varying HESI probe parameters, vaporizer temperature and gas settings were found to have negligible impact on CV optima across the ranges investigated, with only the spray insert depth position having an impact on CV optimum (shifting values up to 5 V between high (H) and low (L) settings). Here, position M was chosen to offer the best compromise between signal intensity and spread of CV values across the monitored ions. (Data not shown)
Using the optimized source parameters and a flow rate of 260 µL/min, the CV optima listed in Table 3 were determined for the SPLASH LipidoMix lipid standards.

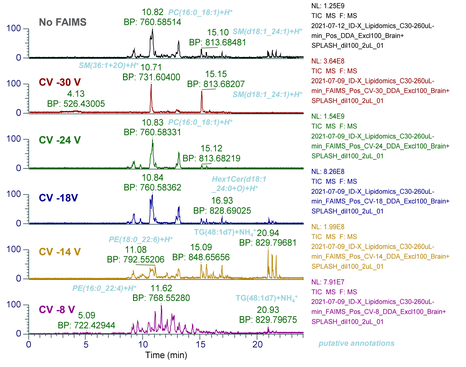
LC-MS analysis of lipid extracts

From the CV optima for the components of the SPLASH LipidoMix standards listed in Table 3, the following CV values were chosen for single-CV LC-MS experiments of the four lipid extract samples to maximize coverage of different lipid classes in a minimum number of run:

Positive ESI mode (V): CV 9, -6, -8, -14, -18, -24, -30
Negative ESI mode (V): CV 24, 29, 30, 33, 35

As shown in Figure 3, the different CV values resulted in gas-phase fractionation of the lipid extracts by lipid class. This allowed higher sensitivity for the detection of particular lipid classes at their CV optima, as shown in Figures 4 and 5, because lower overall ion flux enabled longer injection times.

Figure 3. Total Ion Chromatograms at different CV voltages compared to without FAIMS in positive ESI mode for porcine brain extract, showing selectivity of FAIMS for lipid classes.



Reduction of precursor interference with FAIMS Pro Duo interface

Alongside the increase in S/N, an additional benefit of the selectivity of FAIMS separation was seen in the distinction of isobaric lipid species that were chromatographically unresolved, as shown in Figures 6 and 7.

Figure 4. Difference in MS1 spectra without FAIMS compared to CV –18 V and –8 V, for the magnified mass range of 700-850 Da at RT 9.17 min in the analysis of porcine brain extract in positive ESI mode, showing selective detection of ions at the different CV voltages.

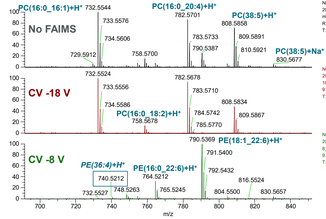


Figure 5. Difference in MS1 signal-to-noise value between measurement without FAIMS and at CV –8V for PE(36:4)+H+ (*m/z* 740.52245) at RT 9.17 in porcine brain extract.

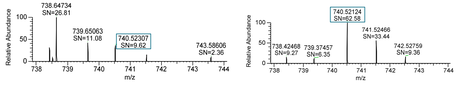


Figure 6. Example of co-eluting isobaric lipid species PS(36:2)+Na+ (*m/z* 510.5256) and PC(38:4)+H+ (*m/z* 510.6007), separated by FAIMS Pro Duo at different CV values, as shown at the MS1 level at RT 10.3 min in positive ESI mode in Heart total lipid extract.

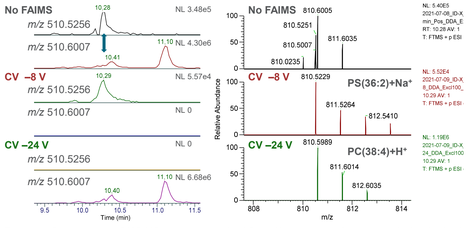


Figure 7. Comparison of MS/MS spectra acquired from Heart extract for the co-eluting isobaric lipids shown in Figure 5, showing the chimeric MS/MS spectrum of the two species without FAIMS (top), and the fragmentation spectra obtained without precursor co-isolation at CV –8 V and –24 V resulting in higher-confidence lipid annotation.

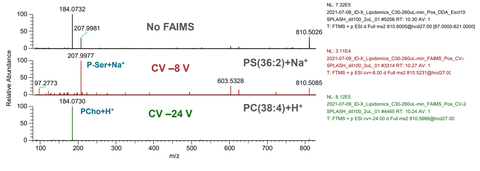
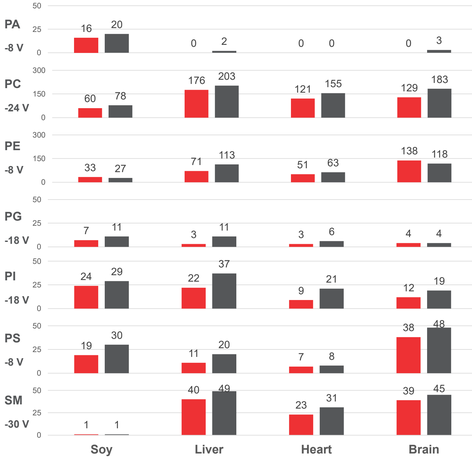


Figure 8. Summary of selected phospholipid classes showing increase in lipid annotations from LipidSearch 4.2 at the respective optimal CV values (gray) compared to 'no FAIMS' (red) in positive ESI mode, as a result of the gas-phase fractionation allowing MS/MS sampling of lower abundant lipid species of the respective lipid classes.



CONCLUSIONS

- Differentiation of individual lipid classes and adducts was achieved at analytical LC flow rates using the FAIMS Pro Duo interface.
- The selectivity of the FAIMS Pro Duo allowed more sensitive precursor detection (increased S/N) and resolution of isobaric lipid species for improved annotation confidence (removing MS/MS interference), as demonstrated across multiple sample matrices.
- Experiments utilizing CV switching may provide broader coverage of different lipid classes to increase the utility of this approach beyond targeting of specific lipid classes in the future.

REFERENCES

1. Purves, R.W.; Prasad, S.; Belford, M. *et. al. J. Am. Soc. Mass Spectrom.* **2017**, *28*, 525–538. (doi.org/10.1007/s13361-016-1587-6)
2. Wei, M.S., Kemperman, R.H.J. & Yost, R.A. *J. Am. Soc. Mass Spectrom.* **2019**, *30*, 731–742 (2019). (doi.org/10.1007/s13361-019-02175-w)

TRADEMARKS/LICENSING

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